

EFFECT OF D-GLUCOSE ON THE INCORPORATION OF ^{32}P INTO PHOSPHOLIPIDS OF MOUSE PANCREATIC ISLETS

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1. Introduction

Exposure of β -cells to D-glucose induces a prompt discharge of insulin and stimulates the synthesis of proinsulin and the formation of new secretory granules [1]. These processes engage β -cell membranes, and as phospholipids are integral parts of all cell membranes, it seemed possible that insulin secretion might be accompanied by an alteration in the metabolism of membrane phospholipids. We have therefore studied the composition of, and the incorporation of ^{32}P -orthophosphate into, phospholipids of isolated pancreatic islets from obese-hyperglycemic mice. These islets consist to more than 90% of β -cells [2], whose secretory pattern in response to D-glucose is well known [3].

We found that the islets contained 115 nmoles phospholipid per mg dry weight, with phosphatidyl choline as the major component. Insulin release, but not the incorporation of ^{32}P into islet phospholipids, was stimulated when islets were exposed to 20 mM D-glucose for 5 min. However, after 60 and 120 min incubation, the stimulated insulin release was accompanied by an increased ^{32}P -labelling of the islets phospholipids. The combined phosphatidyl inositol/phosphatidyl serine fraction incorporated most of the label. These experiments suggest that D-glucose stimulates the metabolism of β -cell phospholipids, predominantly the acidic phospholipids phosphatidyl inositol and/or phosphatidyl serine.

2. Material and methods

2.1. *Animals and incubation of islets*

Adult female obese-hyperglycemic mice, taken from a local colony [2], were fasted overnight and killed by decapitation. Pancreatic islets were isolated and incubated as described in detail elsewhere [3]. The islets were microdissected freehand at room temperature in gassed ($\text{O}_2\text{--CO}_2$, 95:5) Krebs-Ringer bicarbonate buffer [4]. After preincubation for 40 min at 37° in 515 μl bicarbonate buffer containing 100–150 μCi carrier-free ^{32}P -orthophosphate (The Radiochemical Centre, Amersham, England), batches of two islets were incubated for various time intervals in 315 μl bicarbonate buffer or without 100–150 μCi ^{32}P -orthophosphate. The details of the experiments are given in the legends to the figures and tables. The incubated islets were placed on aluminium foil and freed of contaminating fluid with the aid of a micropipette. They were then immediately dropped into isopentane chilled to its freezing point by liquid nitrogen. After freeze-drying (-40° , 0.001 mm Hg) overnight, the islets were weighed on a quartz fiber balance [5].

2.2. *Measurements of insulin*

To remove ^{32}P , which interfered with the counting of [^{125}I]insulin, the proteins in all incubation media were precipitated with 10% TCA (w/v in final concentration). The precipitates, washed once with 10% TCA (w/v), were dissolved in 40 mM phosphate buffer, pH 7.4, containing 0.5% human serum albumin (AB Kabi, Stockholm, Sweden) and 0.9% NaCl. Insulin was then determined by radioimmunoassay; free and antibody-bound insulin were separated by ethanol

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precipitation [6]. Insulin antibodies were from the Radiochemical Centre, Amersham, England, and [^{125}I]insulin from Farbwerke Hoechst AG, Frankfurt/M, Germany. Crystalline mouse insulin prepared by Novo A/S, Copenhagen, Denmark, was used as standard.

2.3. Extraction and separation of phospholipid classes

The freeze-dried islets were transferred to tubes containing 5 ml of chloroform:methanol 2:1 (v/v) and allowed to stand at room temp for 2–4 hr to extract the lipids. Tubes containing only solvent served as controls. Carrier phospholipids (from rat liver) dissolved in chloroform were then added, followed by 2 ml 0.9% (w/v) NaCl. The tubes were shaken and allowed to stand overnight. The upper phases were discarded and the chloroform phases, containing the lipids, were taken to dryness in a stream of nitrogen. The lipids were then dissolved in 0.5 ml chloroform. The tubes were gassed with nitrogen, sealed and stored at -18° until analyzed. Aliquots of the lipids extracts were spotted on 20×20 cm thin layer plates coated with a 0.5 mm layer of Silica gel H (AB Merck, Germany). After chromatographic separation in the solvent system of Skipski [7], the spots were visualized by spraying the plates with 1% (w/v) iodine in methanol. The spots were scraped off into counting vials, 10 ml of scintillation fluid (3 g 2,5-diphenyloxazole and 100 mg 1,4-bis 2-(methyl-5-phenyloxazylyl) benzene per litre toluene:methanol 9:1 (v/v) was added, and the radioactivity was measured in a Packard Model 3320 liquid scintillation spectrometer. Model experiments showed that this procedure gave more than 90% recovery. For determination of islet phospholipid composition, phospholipids from 18 mg freeze-dried islets were extracted with chloroform:methanol and fractionated by thin-layer chromatography as described above. The spots corresponding to the individual phospholipid fractions were scraped off and the phospholipids eluted according to Arvidson [8]. Lipid phosphorus was determined according to Chen et al. [9].

3. Results

The freeze-dried mouse islets contained 115 nmoles phospholipids per mg dry weight. These comprised 56% phosphatidyl choline, 12% phosphatidyl inositol/phosphatidyl serine, 13% phosphatidyl glycerol/phosphatidic acid, 11% phosphatidyl ethanolamine and 8% sphingomyelin. The recovery of lipid phosphorus from the chromatoplates in three separate experiments was about 80%. Two series of experiments

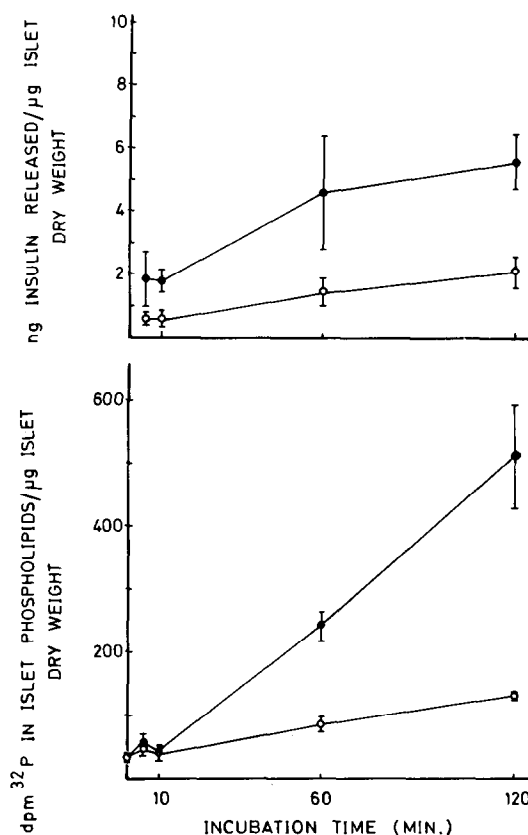


Fig. 1. Effect of glucose on incorporation of ^{32}P into total islet phospholipids. Isolated islets were preincubated for 40 min in 515 μl glucose-free Krebs-Ringer bicarbonate buffer containing 100 μCi ^{32}P and then incubated for 5, 10, 60 or 120 min in 315 μl buffer containing 100 μCi ^{32}P with (●—●) or without (○—○) 20 mM D-glucose. Mean values \pm S.E.M. for 3 experiments.

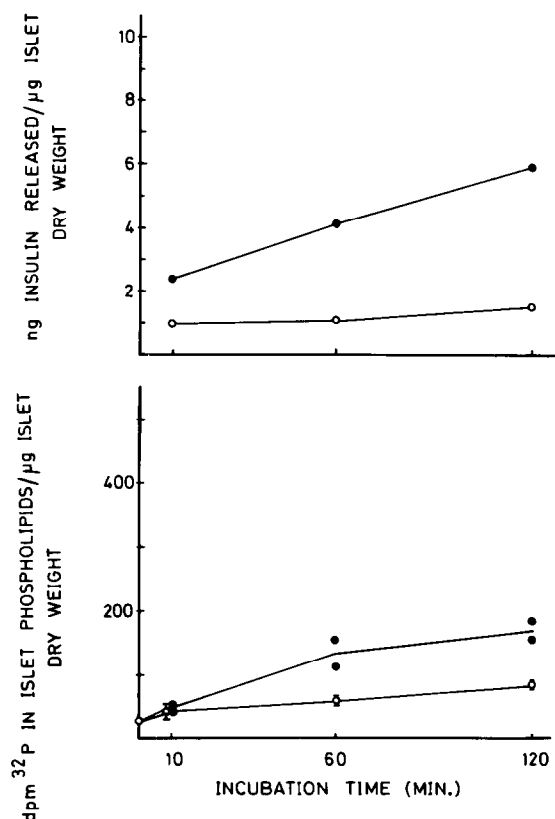


Fig. 2. Effect of glucose on incorporation of ^{32}P into total islet phospholipids. Isolated islets were preincubated for 40 min in 515 μl Krebs-Ringer bicarbonate buffer containing 3 mM glucose and 150 mCi ^{32}P . The medium was then sucked off and the islets were washed once with 500 μl buffer before incubation for 10, 60 or 120 min in 315 μl buffer containing 3 mM ($\circ-\circ-\circ$) or 20 mM ($\bullet-\bullet-\bullet$) D-glucose but no ^{32}P . Mean values \pm S.E.M. for 2–3 experiments.

were performed to study the incorporation of ^{32}P -orthophosphate into phospholipids of isolated islets. In the first series (fig. 1) ^{32}P -orthophosphate was present during both the preincubation and incubation periods. In the second series of experiments (fig. 2) ^{32}P -orthophosphate was present during the preincubation period only and the islets were thereafter washed once with 500 μl Krebs-Ringer bicarbonate buffer without ^{32}P -orthophosphate before starting the incubation period. At all time intervals studied 20 mM D-glucose stimulated the release of insulin (figs. 1 and 2). As is apparent from the figures,

exposure of the islets to 20 mM D-glucose for 5 or 10 min did not change the amounts of ^{32}P recovered in the total islet phospholipids. However, after 60 or 120 min incubation there was a marked increase in the ^{32}P -labelling of the phospholipids. The observed effect was more pronounced when ^{32}P -orthophosphate was present during the incubation period (fig. 1) than when it was omitted (fig. 2).

The distribution of ^{32}P radioactivity among islet phospholipid fractions in the two series of experiments is shown in table 1. The combined phosphatidyl inositol/phosphatidyl serine fraction was the most heavily labelled. The incorporation of ^{32}P into this fraction was significantly enhanced by 20 mM D-glucose in both absolute and relative terms, whereas the ^{32}P -labelling of other phospholipid fractions was only slightly enhanced.

4. Discussion

Since cellular membranes are involved in the transport, storage and release of insulin, stimulation of insulin secretion may in some way affect the metabolism of membrane constituents such as the phospholipids. Studies on secretory cells have indicated that stimulation of secretion is associated with an increased turnover of certain phospholipids [10–12].

Insulin is released within minutes when β -cells are exposed to a glucose stimulus [1]. In our system, there was a significant release of insulin in response to 20 mM D-glucose after 5 min of incubation. However, the incorporation of ^{32}P into islet phospholipids was not enhanced during the first 5 min of glucose-stimulated insulin release, but only after 60 and 120 min. This suggests that the "phospholipid effect" [10] may be related to a later phase of glucose-induced insulin release rather than to the immediate response. A similar time course of incorporation of ^{32}P into phospholipids was observed when the release of catecholamine from adrenal medullary slices was stimulated with acetylcholine [11].

Stimulation of various secretory cells mainly result in an increase in the ^{32}P -labelling of phosphatidyl inositol [10–12]. Similarly, we found that the combined phosphatidyl inositol/phosphatidyl serine fraction incorporated the most ^{32}P when the pancreatic islets were stimulated with D-glucose. It should

Table 1
Incorporation of ^{32}P into islet phospholipid fractions during glucose-stimulated insulin release.

Addition	Incubation time (min)	dpm ^{32}P per fraction* and μg islet dry weight				
		"Front"	PE	PI+PS	PC	"Origin"

<i>Experiment 1</i>						
None	—** (9)	1	4	14	9	6
None	5 (3)	1	9	17	13	9
	10 (3)	2	5	17	12	6
	60 (3)	2	17	40	20	7
	120	2	23	74	26	6
20 mM glucose	5 (3)	1	7	23	12	15
	10 (3)	2	5	23	9	6
	60 (3)	3	19	171	34	13
	120 (3)	6	57	365	73	16
<i>Experiment 2</i>						
None	—** (4)	2	4	10	9	6
3 mM glucose	10 (3)	2	4	16	9	9
	60 (3)	2	6	26	15	8
	120 (3)	4	12	37	24	7
	20 mM glucose	10 (2)	3	9	21	10
60 (2)		2	18	67	35	12
120 (2)		3	22	103	33	11

Experimental details as in fig. 1 (experiment 1) and 2 (experiment 2). The values given in parentheses are the means of the numbers of separate experiments.

* "Front" includes all material moving with the solvent front, e.g. phosphatic acid and cardiolipin. "Origin" includes spingomyelin, lysophosphatidyl choline and, if present, polyphosphoinositides. PE = phosphatidyl ethanolamine; PI = phosphatidyl inositol; PS = phosphatidyl serine; PC = phosphatidyl choline.

** Preincubation only.

be noted that this fraction represents only 12% of the total islet phospholipids. Phosphatidyl inositol comprises about 10% of the total phospholipid of the pancreas. Furthermore, phosphatidyl inositol as well as phosphatidyl serine seem to occur in most cellular membranes without being particularly concentrated to any membrane system [10].

The enhanced incorporation of ^{32}P into islet phospholipids in response to D-glucose does not necessarily mean that there is a true increase in the turnover of islet phospholipids. Increased labelling of phospholipids could be due to alterations in the specific radioactivity of phosphate in islet ATP. Extracellular P_i exchanges slowly with intracellular P_i [13, 14] and an increased rate of exchange might occur in response to D-glucose. The increased rate of glucose utilization in the stimulation in the stimulated

islet might also increase the specific radioactivity of intracellular ATP. However, the fact that there were both absolute and relative increases in the radioactivity of the β -cell phosphatidyl inositol/phosphatidyl serine fraction after D-glucose stimulation suggests that there are specific changes in the metabolism of these phospholipids.

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